



## Bacteriological Profile Of Water Sample And Selected Fish Species From Lower River Benue, Nigeria

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### ABSTRACT

Bacteriological profile of water sample and two freshwater fish species (*Bagrus bayad* and *Oreochromis niloticus*) from four points along Lower River Benue were carried out using primary isolation and biochemical analysis. Total viable bacteria count and total coliform count of the water range from  $4.7 \times 10^6$  to  $8.6 \times 10^6$  CFU/ml and  $3.6 \times 10^4$  to  $5.4 \times 10^4$  CFU/ml respectively. Fish has total viable bacteria count and total coliform count ranges from  $7.20 \times 10^4$  to  $3.20 \times 10^5$  and  $2.40 \times 10^4$  to  $3.12 \times 10^5$ , respectively. Bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp, were found in the water sample while *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* spp, *Staphylococcus epidermis*, *Enterobacter aerogenes* were found in the fish sample.

### INTRODUCTION

Water bodies are economically important to the existence of all living organism as the livelihood of many communities is dependent on the water bodies around them (Yongendra and Puttaiah, 2008). Urbanization and rapidly growing human population results in an increase in waste water discharge into freshwater bodies (Tanimu et al., 2011).

Rivers are the most important freshwater resource for man. Unfortunately, they are being polluted. Pollution of the aquatic environment is a serious and growing problem because it affects the physic chemical characteristics and microbiological quality of the river (Koshy and Nayar, 1999). Owing to the large quantity of effluent discharged into the receiving waters, the natural processes of pathogen reduction are inadequately for protection of public health. In addition, industrial wastes that alter the water pH and provide excessive bacterial nutrients often compromise the ability of the natural processes to inactivate and destroy pathogens. The extent of discharge of domestic and industrial effluents is such that rivers receiving untreated effluents cannot provide the dilution necessary for their survival as good quality water sources. Disposal of sewage wastes into a large volume of water could increase the biological oxygen demands to such a high level that all the available oxygen may be removed, consequently causing death of all aerobic species like fish. Prevention of river pollution requires effective monitoring of physic chemical and microbiological parameters.

In most countries, the principal risks to human health associated with the consumption of polluted water are microbiological in nature. The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health (APHA, 1981). Biological pollutant may also be transferred to human beings through fish consumption (Agarwal, 2005). Aside from human health concerns, the indiscriminate dumping of waste into water bodies can over tax the self purifying capacity of the receiving water. This will not only endanger aquatic lives but also impair other amenity purposes and non consumption uses that the river course might be put in.

River Benue is a major river of economic, agricultural and environmental significance in Makurdi, the capital of Benue state, Nigeria. Its proximity in the town makes it an attractive source of water. Its watershed is intensively cultivated for cereals and vegetable crops. The river receives effluents from water board authority, domestic waste from Wurukum abattoir and Wadata market, and the Benue breweries all located along its course. Riparian activities such as washing, bathing etc are also carried out in the river and around its banks. As a natural water body, River Benue contains wide variety of microbial flora e.g *Aeromonas hydrophila*, *Escherichia coli*, *Salmonella typhi*, *Shigella* species and a host of others originating from living and non-living animals. Some of this microorganism maybe disease causing organisms to aquatic lives and to fish in particular (Cheesebrough 2006; Agarwal 2005). The lack of good and modern sanitary waste treatment system for the town and the presence of many abandoned waste sites along the river lane make it the final receptor of an extremely complex and uncontrolled drainage system.

It has been reported that the major identified source of pollution of river Benue was the direct runoff of effluents from industries and domestic sources. Therefore the river is exposed to high level of eutrophication due to organic matter and industrial effluents discharged into it. This informs the need to evaluate the profile of bacteria in the water body and in the fish. The objective of this research is to investigate the bacteriological content of water sample from River Benue and two selected fish species. To isolate and identify potential pathogenic organism in the water and fish sample collected from the lower River Benue.

### MATERIALS AND METHODS

#### Sampling stations

Four sampling stations were mapped out along the Lower River Benue within the Makurdi metropolitan area for the study as thus;

**Site I (Brewery Station):** The brewery is located 5km away from Makurdi town along Makurdi-Gboko road. Brewery produces effluents from its daily routine production. The effluents are channelled into Ageba, a tributary of River Benue and it flows into the River.

**Site II (Wurukum Abattoir Station):** This station is about 5m from Wadata market

**Site III (Water Works Station):** This is about 1.5km away from Wadata market and about 200m away from Makurdi water works. It receives discharge from Makurdi water works.

**Site IV (Wadata Market Station):** Wadata market is located on the bank of River Benue. This station receives municipal wastes that compose of solid wastes, abattoir effluents and domestic wastes generated from the market. A huge heap of refuse dump is found at this location where its wastes are leached directly into the river.

#### Sample collection

Water samples for bacteriological study were collected by dipping the sample bottle into the river. The bottle was opened, allowed to fill up with water then corked while still under water (APHA, 1998). This was labeled and kept in ice and taken to the laboratory for bacteriological analysis. Fish samples were collected in a sterile container and this was covered and also kept in ice before taken to the laboratory for microbial analysis.

#### Microbial analysis

Primary isolation and biochemical analysis was done on the isolate to identify the pathogen present in the water and fish samples.

#### Sample preparation

The fishes were cut in bits, grinded and 1g was measured out. 9ml of sterile normal saline was dispensed into sterile test tube and

respectively of water and the fish sample was suspended in the 9 ml of sterile normal saline. A serial dilution of each of these homogenates was prepared. A 1ml aliquot of the serially diluted homogenate was inoculated in a petri dish containing sterile Nutrient agar and Macconkey agar (for total viable count and total coliform count respectively) and swirled clockwise and anti-clockwise to ensure even mixture of the medium and the inoculums as earlier done by Ogbondeminu et al., (1991) and Jones (1979). The plates were allowed to gel for about 30 minutes after which they were wrapped together in aluminium foil and incubated at 37C for 24 hours.

After incubation, the plates were viewed and discrete colonies were counted and the colony forming unit was calculated using

Colony forming unit = number of colonies on the plate x dilution factor

$$\frac{\text{Number of colonies on plates}}{\text{Volume inoculums (ml)}}$$

Where,

Number of colonies on plates = counted colonies on plates

Volume of inoculums = 1ml

Dilution factor = 10-3 and 10-5

After the colony counts, representative colonies were sub-cultured on sterile Nutrient agar plates and incubated for 18-24 at 37C from where pure cultures were saved in sterile agar slants at 4C for characterization.

#### Identification and Characterization of the Isolate

All isolates were sub-cultured to obtain a pure culture and a gram-staining carried out. Identification of the isolates was carried out based on the method described by Sakazaki and Shimad (1986), Collins et. al., (1989) and Cheesebrough (2006).

## RESULTS

### Bacterial enumeration of water samples from the four sampling

**Table 1:** Bacterial enumeration of water from the different sampling points

S/no	Sampling points	TVC (CFU/ml)	TCC (CFU/ml)
1	Brewery	$5.7 \times 10^6 \pm 0.00^b$	$3.8 \times 10^4 \pm 0.00^b$
2	Wurukum	$7.6 \times 10^6 \pm 0.00^c$	$4.6 \times 10^4 \pm 0.00^c$
3	Wadata	$8.6 \times 10^6 \pm 0.00^d$	$5.4 \times 10^4 \pm 0.00^d$
4	Water works	$4.7 \times 10^6 \pm 0.00^a$	$3.6 \times 10^4 \pm 0.00^a$

**Table 2:** Bacteria enumeration of fish sample

S/no	Sampling points	Fish species	TVC (CFU/ml)	TCC (CFU/ml)
1	Brewery	<i>Bagrus bayad</i>	$7.20 \times 10^4 \pm 0.00^a$	$3.12 \times 10^5 \pm 0.00^a$
	“	<i>Oreochromis niloticus</i>	$3.04 \times 10^5 \pm 0.00^a$	$2.40 \times 10^4 \pm 0.00^a$
2	Wurukum	<i>Bagrus bayad</i>	$2.96 \times 10^5 \pm 0.00^a$	$1.04 \times 10^5 \pm 0.00^a$
	“	<i>Oreochromis niloticus</i>	$2.82 \times 10^5 \pm 0.00^a$	$1.24 \times 10^5 \pm 0.00^a$
3	Wadata	<i>Bagrus bayad</i>	$3.20 \times 10^5 \pm 0.00^a$	$2.02 \times 10^5 \pm 0.00^a$
		<i>Oreochromis niloticus</i>	$2.10 \times 10^5 \pm 0.00^a$	$2.04 \times 10^5 \pm 0.00^a$
4	Water works	<i>Bagrus bayad</i>	$2.60 \times 10^5 \pm 0.00^a$	$1.08 \times 10^5 \pm 0.00^a$
		<i>Oreochromis niloticus</i>	$1.88 \times 10^5 \pm 0.00^a$	$1.28 \times 10^5 \pm 0.00^a$

### points

Results of the bacterial enumeration of the water samples as shown in Table 1 reveals that Wadata station had the highest microbial load count ( $8.6 \times 10^6$  and  $5.4 \times 10^4$  CFU/ml) for total viable count and total coliform count while water work had the lowest microbial load ( $4.7 \times 10^6$  and  $3.6 \times 10^4$  CFU/ml) for total viable and total coliform count respectively.

### Bacterial enumeration of fish samples from the four sampling points

The results of the bacteria enumeration of the fish samples from the sampling stations shown in Table 2 reveals that Bagrus bayad collected at the Wadata station has the highest microbial count ( $3.2 \times 10^5$  and  $2.02 \times 10^5$ ) for total viable count and total coliform count respectively, and the order increases from Wurukum Water works Brewery respectively with Brewery station having the lowest count for total viable count and total coliform count ( $7.2 \times 10^4$  and  $1.02 \times 10^4$  CFU/g).

For *Oreochromis niloticus*, total viable count was highest at Brewery station ( $3.04 \times 10^5$ ) and lowest at Wadata station ( $2.10 \times 10^5$ ) and total coliform count was lowest at Brewery station ( $2.4 \times 10^4$ ), and increases from Wurukum to water works and Wadata stations respectively with Wadata having the highest ( $2.04 \times 10^5$ ).

### Biochemical characterization from water samples

Results of the biochemical characterization from water sample from the sampling station is shown in table 3. Out of the 23 isolates, *E. coli*, *Staphylococcus aureus* were four in number each, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were 3 each, *Shigella* spp., *Salmonella* spp., *Bacillus cereus* were two each and others such as occurring once.

### Biochemical characterization from fish samples

Results of the biochemical characterization from water sample from the sampling station is shown in table 4. Out of the 20 isolates, *E. coli*, was four in number, *Staphylococcus cereus* was 3, *Shigella* spp., *Bacillus cereus* and others occurring twice.

Table 3: Biochemical characterization from water sample.

Isolate	Color of colony	Gram stain	Shape	Catalase	Coagulase	Indole	Citrate	Urease	Oxidase	Organism
1	Green metallic sheen	-	Rod	+	NA	+	-	-	-	E.c
2	Grayish	+	Rod	+	NA	-	+	-	+	B.c
3	N Pale	-	Rod	+	NA	-	+	-	+	P.a
4	Pink	+	Cocin chain	-	NA	-	-	-	-	En. F
5	Creamy	+	Cocci in cluster	+	+	-	-	-	-	St.a
6	Creamy	-	Rod	+	NA	-	+	-	-	Ent.a
7	Pale	-	Rod	+	NA	-	+	+	-	K .spp
8	Pale	-	Rod	+	NA	-	+	-	-	S .spp
9	Creamy	+	Cocci in cluster	+	+	-	-	-	-	St. a
10	Pale	-	Rod	+	NA	-	+	-	+	P.a
11	Pale	-	Rod	+	NA	-	-	-	-	Sh.spp
12	Green metallic sheen	-	Rod	+	NA	+	-	-	-	E.c
13	Pink	+	Cocci in cluster	-	NA	-	-	-	-	En.f
14	Pale	-	Rod	+	NA	-	+	-	-	S.spp
15	Green metallic sheen	-	Rod	+	NA	+	-	-	-	E.c
16	Pale	-	Rod	+	NA	-	+	-	+	P.a
17	Pale	-	Rod	+	NA	-	-	-	-	Sh.spp
18	Green metallic sheen	-	Rod	+	NA	+	-	-	-	E.c
19	Grayish	+	Rod	+	NA	-	+	-	+	B.c
20	Pink	+	Cocci in cluster	-	NA	-	-	-	-	En.f
21	Creamy	+	Cocci in cluster	+	-	-	-	-	-	St.e
22	Creamy	+	Cocci in cluster	+	+	-	-	-	-	St.a
23	Creamy	+	Cocci	+	+	-	-	-	-	St.a

Key; NA-----not application, (-) negative, (+) positive; E.c *Escherichia coli*; B.c *Bacillus cereus*; St.a *Staphylococcus aureus*; St.e *Staphylococcus epidermis*; En.f *Enterococcus faecalis*; K.spp *Klebsiella* spp; S.spp *Salmonella* spp; Sh.spp *Shigella* spp; P.a *Pseudomonas aeruginosa*; Ent.a *Enterobacter aerogenes*

## DISCUSSION

The analysis of bacteria isolates in the water and fish reveals a lower load of bacteria at the Brewery station compare to the Wurukum and Wadata station. This could be due to the much anthropogenic activities that is being carried out there. The difference in the bacterial load agrees with the fact that the presence of bacteria in natural aquatic ecosystem is dependent upon the rate of contamination and the equilibrium that is established between the bacterial proliferations in the environment and the rate of its elimination (Lejeune et al., 2001).

Total coliform count in all the sampling point was higher than that of WHO (1992) standard ( $1.0 \times 10^3$ CFU/100ml). This may be because most people in this part of the world see Rivers as a dumping ground for every kind of waste (Atribom et al., 2014). Fish samples were seen to harbor *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp, *Pseudomonas aeruginosa*. This result agrees with Mitchell (1972) and Atribom et al, (2014), who reported that fresh water fishes may harbor human pathogens after exposure to contaminated water or food sources.

Although most of these bacteria are found very useful in every living environment, but they become harmful and pathogenic when they

exceeds their average limits

The alarming high number of total coli forms and thermo tolerant (faecal) coli form per 100ml obtained from the water samples, which exceeds at least ten times the recommended limit, indicates high level of faecal pollution of the river water which potentially poses a high health risk for recreational purpose. This clearly implies that the organic pollution of the water is of faecal pollution (Olatunji et al., 2011).

## CONCLUSION

Bacteriological analysis of the river at the various sampling points revealed high load of bacteria which are known to be pathogenic to both fish and humans. Total viable bacteria count and total coliform count of the water ranged from  $4.7 \times 10^6$  to  $8.6 \times 10^6$  CFU/ml and  $3.6 \times 10^4$  to  $5.4 \times 10^4$  CFU/ml respectively. Fish had total viable bacteria count and total coliform count ranged from  $7.20 \times 10^4$  to  $3.20 \times 10^5$  and  $2.40 \times 10^4$  to  $3.12 \times 10^5$ , respectively. Bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp, were found in the water sample while *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* spp, *Staphylococcus epidermis*, *Enterobacter aerogenes* were found in the fish sample.

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